LACTIC ACID FROM POTATOES

T. C. CORDON, R. H. TREADWAY, MARGARET D. WALSH, AND MADELYN F. OSBORNE
Eastern Regional Research Laboratory, Philadelphia 18, Pa.

In a search for additional industrial products that can be made from cull and surplus potatoes, preparation of lactic acid from potatoes was investigated. Fungal amylases (*Aspergillus niger* N.R.R.L. 330 in most cases) were used to convert the starch of the potato to sugars, and three strains of *Lactobacilli* were evaluated for the fermentation. Potato without added nutrients was used as the culturing medium for both *A. niger* and the *Lactobacilli*. The acid was recovered and purified as methyl lactate by the methanol vapor method. An average of 85.5% of the lactic acid in the mash was recovered.

POTATO surpluses in recent years emphasize the need for new means of utilizing cull and surplus potatoes. Culls are used regularly for manufacture of starch, and surplus potatoes have been used for production of alcohol. Potatoes are an excellent substrate for the growth of microorganisms, because they possess valuable carbohydrate, protein, and mineral constituents. Potato water has been used as a supplement in making penicillin in Germany. The I. G. Farbenindustrie report (*14*) states that the best supplementary substance found was potato water; even after long standing, it retained its activity as a supplementary substance.

Production of lactic acid from potatoes is apparently not reported in the literature, although potato starch has provided the raw material for manufacture of lactic acid in Germany. Scientists who investigated the German chemical industry at the close of World War II reported that either potato starch or beet sugar was used as raw material for lactic acid fermentation (*3*). Either malt or sulfuric acid was used to convert starch to sugar before acid fermentation. At present, lactic acid is manufactured in the United States (*21*) by fermentation of dextrose, lactose, or sirups such as invert sirup. Nutrients such as ammonium sulfate are added to supply inorganic requirements, and malt sprouts or protein concentrates are used to supply organic nutrients.

This study was undertaken to determine whether amylases produced by the submerged growth of *Aspergillus niger* could convert starch in potato to fermentable sugars efficiently and whether the hydrolyzate could be fermented to lactic acid, a chemical having many potential industrial applications (*11*).

Fungal amylases are prepared either by propagation of the fungus on the surface of the substrate or in submerged culture. Although fungal amylases have been used for centuries in China and Japan, Takamine (*25*) is credited with first suggesting in this country the commercial use of mold amylase for converting starch.

Underkofler and collaborators (*27–29*) have done considerable work on production of mold bran. Hao and Jump (*13*) and Roberts and co-workers (*22*) have reported the use of mold bran in hydrolyzing starch.

Recently, submerged culture of amylase-producing molds has received considerable attention. Workers at the Northern Regional Research Laboratory (*4*, *5*, *16*, *26*, *30*) have described these methods and use of the enzymes produced.

Adams *et al.* (*1*), Erb *et al.* (*8*), Pan *et al.* (*20*), and Lipps *et al.* (*18*) have studied methods of using *A. niger* N.R.R.L. 337 to produce mold amylases.

EXPERIMENTAL

PREPARATION OF POTATO MASH. Whole washed potatoes were ground in a hammer mill. Because of the consistency of cooked potato, water was added before cooking. In the first experiments, the added water amounted to about one half the weight

Table I. Effects of Various Treatments with *A. niger* N.R.R.L. 330 on Saccharification and Fermentation by *L. delbrückii* N.R.R.L.-B-445

(Moisture- and ash-free basis)

Expt. No.	Inoculum or Enzyme Source	Saccharification: Maltase units	Saccharification: Treatment[a]	Fermentation Time, Hours	Residual Soluble Carbohydrate (As Dextrose), %[b]	Residual Reducing Sugar (As Dextrose), %[b]	Yield of Lactic Acid, %: Based on organic material	Yield of Lactic Acid, %: Based on original carbohydrate	Yield of Lactic Acid, %: Based sugar consumed
9	Spore suspension	26[c]	Aerated for 72 hours at 30° C.	88	26.1	9.9	25.0	30.3	44.4
6	1.6%[d] culture liquor	75 194[e]	Aerated for 44 hours at 30° C.	99	6.5	1.1	49.3	66.0	72.0
12	5.0% culture liquor	113	Held for 1.5 hours at 45° C.	137	19.4	8.6	54.1	64.5	83.9
1	22.5% culture	45	Held for 2 hours at 60° C.	138	19.4	7.4	53.6	73.5	92.7

[a] Before inoculation with *L. delbrückii* and incubation at 45°.
[b] At end of fermentation.
[c] Measured after 44 hours incubation.
[d] Based on volume of mash.
[e] Measured after 60 hours of lactic acid fermentation.

of potato. In later experiments, to prevent crystallization of the calcium lactate produced during fermentation, the water-potato ratio was increased to 1:1, giving 6 to 7% sugars on complete hydrolysis. Also, to further thin the mash and facilitate mixing, 0.5 to 1% mold culture liquor (based on weight of mash) was added before heating.

Several methods of cooking were investigated. Heating in an autoclave at 125° to 135° C. for 1 hour with constant stirring, followed by aseptic transfer to a fermentor, was quite satisfactory. It was found, however, that pasting ground potato at 100° C. with stirring, then transferring to the fermentor and autoclaving for 1 hour at 130° C. or 2 hours at 120° C. was adequate treatment for conversion of the starch to sugars in the subsequent saccharification. Although cooking at high temperature is necessary for sterilization, it appears to be unnecessary for conversion and fermentation. In one experiment, the average yield of lactic acid was obtained by merely steaming the ground potatoes for 0.5 hour.

Production of Amylase and Methods of Use. Amylases produced by submerged growth of *Aspergillus niger* N.R.R.L. 330 or 337 were used for conversion of starch to sugars. Procedures developed at the Northern Regional Research Laboratory (*16*) were used for growing the molds.

In preliminary experiments, the distillers' thin stillage-dextrose medium recommended by Le Mense *et al.* (*16*) was used. More enzyme was obtained, however, by using whole cooked potato thinned with mold enzyme or saccharified with mold enzyme and diluted to about 5% solids content than with any of the other mediums tried. These included (*a*) reconstituted grain (half corn and half milo) thin stillage, (*b*) potato thin stillage, (*c*) fresh grain thin stillage, and (*d*) $^2/_3$ potato and $^1/_3$ molasses thin stillage, each supplemented with 2% dextrose and 0.5% calcium carbonate. (Since this work was completed, considerable information has been obtained concerning amylase production by *A. niger* N.R.R.L. 330 on potato mediums.)

Of the two available strains of *A. niger* (N.R.R.L. 337 and N.R.R.L. 330), the latter produced considerably more maltase than the former. These investigations showed that maltase is more important than α-amylase in producing sugars from potatoes for lactic acid fermentation. The maltase enzyme is also better for converting grain starches for alcoholic fermentations (*4*). For this reason, α-amylase potencies were not determined on all cultures used. A number of N.R.R.L. 330 and 337 *A. niger* cultures grown for several days in stillage and in cooked potato mediums produced amylase potencies which ranged from 0.3 to 13 α-amylase units. These values are of the same magnitude obtained by other workers. Since potent 330 cultures also produce almost instantaneous thinning of cooked potatoes, this organism was used in most of the studies.

Various conditions for utilizing the enzymes were investigated. In some cases, cooked potato slurry was inoculated with 1.5 to 2.5% submerged culture; in others, mold spores were used. The enzymes were then produced in situ in 1 to 3 days. In other cases, up to 22.5% by volume of submerged culture containing the enzymes was added to the cooked potatoes, and only a short period—0.5 to 2 hours—was allowed for saccharification.

Preparation of *Lactobacillus inocula*. Because potatoes were to be used for the fermentation, potato mediums seemed advisible for culturing the *Lactobacilli*. Finely ground potato diluted 1:1, or 0.5:1, with water, cooked, and saccharified with submerged mold culture was an excellent medium. The cultures were maintained on liquid or agar mediums containing only calcium carbonate in addition to the saccharified potato. For fermentation experiments, the bacteria were grown in deep tubes of the potato broth medium for 16 to 24 hours, then transferred to flasks of a similar medium and again incubated for 16 to 24 hours before the cooked potato slurry was inoculated. In each case, the inoculum was about 10% by volume of the potato slurry.

Fermentation. Three species of *Lactobacilli*—*L. delbrückii*, *L. pentosus*, and an unidentified species—were tested for their ability to convert the potato sugars to lactic acid. Excess calcium carbonate was supplied for neutralization of the lactic acid and maintenance of pH between 5 and 6, the optimum range for these organisms. Two types of fermentors were used. For experiments in which the mold for saccharification was propagated directly in the potato mash before fermentation, a fermentor of the type described by Feustel and Humfeld (*9*), containing about 2 liters of medium, was used. With this fermentor excellent aeration and stirring could be maintained. After the initial saccharification period, the air was turned off, and the stirrer was operated slowly to keep the carbonate in suspension. When aeration was not required, a 4-liter glass bottle, containing about 3 liters of medium and equipped with a stirrer for keeping the carbonate in suspension, was used. The mash was fermented in a constant-temperature incubator or room at the optimum temperature of the organism.

Analytical Methods. The progress of the fermentations was determined by analyses that indicated the consumption of sugars and the formation of lactic acid in the mash as calcium lactate. Lactic acid was determined in a solution of the spent fermentation liquor diluted 1:50, which reduced the lactic acid concentration to a working level and ensured the complete solution of all lactic acid salts. A modification of Friedmann and Graeser's method was used (*12*); the apparatus was adapted from that of Edwards (*7*). The modified apparatus contains a sintered-glass gas diffusion plate in the acetaldehyde–sodium bisulfite absorption chamber. Because of the large dilution, any interfering substances are in such low concentration that removal is unnecessary.

Some of the final samples from the fermentations were tested to determine the presence of acids, other than lactic, havin soluble calcium salts. The spent fermentation liquor wa diluted 1:4, and the solubilized calcium was determined by th double precipitation and oxidation method of Derx and Jans

(6). This method minimizes errors due to oxidizable substances other than oxalates in the test solution and prevents low calcium results caused by the possible precipitation of calcium phosphates. From the amounts (equivalents) of lactic acid and soluble calcium found, the amount of organic acids present other than lactic was obtained by difference.

Starch was determined by the polarimetric method of Steiner and Guthrie (24). Total hydrochloric acid-hydrolyzable carbohydrate, total soluble carbohydrate, sugars, and ash were determined by methods of the Association of Official Agricultural Chemists (2).

Table II. Yields of Lactic Acid Obtained with Different Species of *Lactobacilli*

(Fermentation time, 112 hours)

Expt. No.	Culture	Maltase Units	Fermentation Temp., ° C.	Yields of Lactic Acid, %: Based on organic material	Based on original carbohydrate[a]	Based on sugar consumed
11	*L. delbrückii* N.R.R.L.-B-445	61	45	64.5	79.4	93.8
14	*Lactobacillus*[b]	48	45	52.9	61.9	76.0
15	*L. pentosus* 124-2[c]	75	30	77.1	91.2	95.2
16		62	30	68.9	86.3	91.2
21		70	30	70.4	84.4	87.9

[a] On moisture- and ash-free basis.
[b] Aerobic culture obtained from industrial concern; slow aeration provided during fermentation.
[c] Obtained from W. H. Peterson, University of Wisconsin.

Yields of lactic acid were arrived at in the following manner:

The total fermentable carbohydrate content was taken as the sum of the dextrose equivalents of the starch and total sugars in the potatoes. This value was altered somewhat by the additions of mold amylase and *Lactobacillus* inocula, but attempts to obtain more accurate values by hydrochloric acid hydrolysis of the mash at the beginning of fermentation were unsuccessful. These values were invariably lower than the sum of the starch and sugars.

All calculations were made on the dry, ash-free basis. Yield of lactic acid was calculated as percentage of organic material, percentage of original carbohydrate, and percentage of sugar consumed (original carbohydrate minus residual carbohydrate).

Lactic acid was separated and recovered from the crude fermentation mixture by the methanol vapor method (10). The mash was acidified with concentrated sulfuric acid to pH 1.9 to 2.0 and digested at 100° C. for 2 hours. This treatment produced a readily filterable mixture. The filtrate was evaporated to a concentration of 25 to 50% and then treated with methanol vapor at 100° C. This method resulted in recovery of an aqueous, colorless solution of methyl lactate, essentially free of impurities. Saponification indicated recovery yields of 88, 86, 94, and 74% (four experiments).

In three experiments the butyl ester was prepared from the methyl lactate as outlined in the following typical experiment:

The aqueous methyl lactate (330 ml.) containing 0.63 mole of saponifiable material was heated to remove methanol (vapor temperature to 95° C. at atmospheric pressure). Then butanol (100 ml.) and concentrated sulfuric acid (esterification catalyst, 0.2 ml.) were added and the water removed azeotropically. When all the water had been removed sodium acetate (1 gram) was added to neutralize the catalyst, and the mixture was distilled to give butyl lactate (0.42 mole). Of the saponifiable matter, 57, 64, and 65% were recovered as butyl lactate in the three experiments.

Conversion to butyl lactate, checked by boiling point and index of refraction, was taken as additional proof of the identity of the lactic acid produced in the fermentation.

Although it was not tried in this work, the hot-solvent extraction method of Leonard, Peterson, and Johnson (17) might prove useful in recovery of lactic acid from fermented potato mash.

Enzyme potency determinations were made on the mold culture filtrates. Maltase activity was determined by the method of Corman and Langlykke (4), in which activity units represent the percentage of maltose hydrolyzed under a standard set of conditions; α-amylase activity was determined by the method of Sandstedt, Kneen, and Blish (23) as modified by Olson, Evans, and Dickson (19).

RESULTS AND DISCUSSION

Preliminary experiments showed that amylases produced by *A. niger* N.R.R.L. 330 and 337 fail to convert starch completely to sugar in the absence of fermentation organisms. This is generally true of any amylase; they seldom convert more than 75% of starch to sugar. If the sugar is removed as formed, however, as for instance by yeast, the conversion may then go almost to completion. In these studies, when the sugar was removed by *Lactobacillus*, conversion of the starch was almost complete.

Table I shows the results of experiments in which the mold was grown and the saccharifying enzymes produced directly in the mash before inoculation with *L. delbrückii* and incubation at 45° C. With spore inoculation, reasonably high enzyme potencies were obtained, but only 44% of the sugar consumed was converted to lactic acid, and 38% of the original carbohydrate was used by the mold. When 1.6% culture liquor was used for inoculation, high maltase activity was obtained; 72% of the sugar consumed was converted to lactic acid, and 30% of the original carbohydrate was metabolized by the mold. By using preformed amylases in concentrations of 5 to 22.5%, much more of the sugar consumed was converted to lactic acid. However, the mold continued to produce the amylases, even though the lactic acid fermentation (at 45° C.) was started immediately after addition of the mold culture. Maltase activities of more than 400 units have been found in some of these mashes.

That the incomplete conversion of sugars to lactic acid was not due to incomplete saccharification was shown by the fact that considerable reducing sugar still remained at the end of the fermentations with this organism (Table I, experiments 9 and 12). It appears then that this culture of *L. delbrückii* was unable to use all the available sugar under the conditions of these experiments. Nitrogen and phosphorus as diammonium phosphate added to potato mash in 0.1% concentration, based on the total solids content, failed to increase the yield of lactic acid. No other supplemental nutrients were tried.

Although the yields of lactic acid obtained with *L. delbrückii* were regarded as reasonably good, two other strains of *Lactobacilli* obtained later—*L. pentosus* and an unidentified *Lactobacillus* strain—were evaluated. Table II gives typical results. Maltase activity of the fungal amylase (N.R.R.L. 330) was of the same order in each experiment. The unidentified *Lactobacillus* was less efficient than *L. delbrückii*. *L. pentosus* proved to be the best; it gave yields of up to 91.2 and 95.2% acid, based on the original carbohydrate and sugar consumed, respectively.

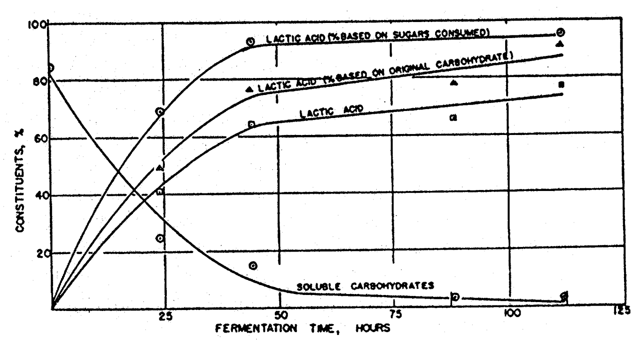

Figure 1. Changes in Lactic Acid and Carbohydrate Content of Potato Mash during Fermentation

Ground potato was diluted with one half its weight of water and cooked; 5% of fungal amylase (N.R.R.L. 330, maltase activity 75 units) was allowed to act for 1 hour; *L. pentosus* inoculum, 10% by volume

Figure 2. Outline of Process for Producing Lactic Acid from Potatoes

Ground whole potatoes + water

↓

Paste (100° C.) in presence of 1% mold culture

↓

Cook and cool

↓

Saccharify (1 hour) with 5% mold culture

↓

Ferment (excess calcium carbonate) with *Lactobacillus* (10% inoculum)

↓

Acidify to pH 1.9 to 2.0 with sulfuric acid

↓

Digest at 100° C. for 2 hours

↓

Filter and concentrate (25 to 50%)

↓

Esterify (100° C.) with methanol vapors

↓

Filter

↓

Distill

↓

Methyl lactate

↓

Add water

↓

Lactic acid and methanol

Yields of 80 to 90%, based on the original carbohydrate, have been obtained consistently with this organism. If 14% is taken as the average starch content of potatoes as marketed and 85% as an average yield of lactic acid, 100 pounds of fresh potatoes would yield about 13 pounds of lactic acid.

Figure 1 shows the progressive changes in lactic acid and carbohydrate during fermentation of potato by *L. pentosus* No. 124-2. This experiment gave the highest yield of lactic acid, although the yields were nearly equaled in some of the other experiments (Table II, experiments 16 and 21). Production of lactic acid was rapid during the first 44 hours; 84% of the total lactic acid was produced in that period. Almost all the carbohydrate was fermented, and nearly all was converted to lactic acid.

In some experiments with *L. pentosus*, 10 to 15% of the original carbohydrate was not accounted for as lactic acid or residual sugar. Lactic acid calculated from the soluble calcium content was much higher than that shown by direct determination, indicating the presence of some other acid. Apparently when sterile (Seitz-filtered) culture filtrate was used for saccharification, the unknown acid was still present. This finding eliminated the mold culture as the source of the other acid. When a solution of dextrose and potato press juice was fermented, about 82% of the original dextrose appeared as lactic acid, and 3.7% remained unfermented. The remainder of the dextrose was not accounted for. The value for lactic acid calculated from the soluble calcium content of the mash agreed well with the value obtained by direct determination. It appears then that although *L. pentosus* usually produces only lactic acid from hexoses (*15*), under certain conditions other acids having soluble calcium salts or other products may also be formed. The conditions under which this occurs have not been defined.

Figure 2 shows in outline form the complete process for converting potato carbohydrate to lactic acid and the esterification process used for recovery.

SUMMARY

Lactic acid was produced from potatoes by using *A. niger* strains N.R.R.L. 330 or 337 to convert the starch to sugars and *Lactobacilli* to ferment the sugars. Yields of lactic acid of 80 to 90% (based on original carbohydrate) were obtained in the fermentation mixture. With the methanol vapor method, an average of 85.5% of the acid in the fermented mash was recovered. Of three organisms studied, *L. pentosus* gave the highest yield.

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